INVERTEBRATES IMPLICATED IN THE TRANSFER OF GAMBIERTOXINS TO THE BENTHIC CARNIVORE *POMADASYS MACULATUS*

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The food chain hypothesis for the transfer of ciguatoxins (CTX) to carnivorous fish has gained widespread acceptance. This study was undertaken to determine the vector(s) transferring gambiertoxins to the often ciguateric blotched javelin fish (*Pomadasys maculatus*) in Platypus Bay, Queensland. *P. maculatus* is a benthic carnivore which in Platypus Bay was found to feed predominantly on small shrimps and crabs that live amongst *Cladophora* sp. that also harbours *Gambierdiscus toxicus*. Of the potential prey of *P. maculatus* in Platypus Bay, only the shrimps (mostly *Alpheus* sp.) contained detectable levels of ciguatoxin-like toxins, implicating shrimps as an important vector in the transfer of gambiertoxins to carnivorous fish. Any toxic effects of *G. toxicus* on shrimps may facilitate the selective feeding of fish on shrimps containing the highest toxin levels. Such selective feeding provides a mechanism for the funnelling of toxins from *G. toxicus* to *P. maculatus*. It remains to be established if shrimps are capable of biotransforming the gambiertoxins to ciguatoxins or whether biotransformation of the gambiertoxins is accomplished exclusively by fish. Given that *P. maculatus* is at times highly toxic, and within a year can be non-toxic, it is likely that the gambiertoxins enter the food chain as intense bursts that perhaps last for only several weeks. Depuration and/or detoxification are likely to account for the apparent rapid loss of gambiertoxins and ciguatoxins from shrimps, crabs and *P. maculatus*.

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The food chain hypothesis for the transfer of ciguatoxins (CTXs) to carnivorous fish has gained widespread acceptance through the results of numerous studies (Randall,1958; Yasumoto et al.,1971,1977a,b,1979; Banner,1974; Murata et al.,1990; Holmes et al.,1991; Lewis et al.,1991; 1992). Key steps in the food chain hypothesis include (i) the uptake by herbivorous fish of gambiertoxins (GTXs) produced by *Gambierdiscus toxicus* and (ii) the transfer of the toxins from herbivorous to carnivorous fish. Grazing molluscs (Yasumoto & Kanno,1976) and fish that feed on invertebrates (Banner,1974) have also been implicated in ciguatera. The involvement of invertebrates in the ciguatera food chain has been speculated upon (Kelly et al.,1992) following laboratory observations that brine shrimp were capable of feeding on *G. toxicus*. However, evidence that invertebrates play an important role in the transfer of CTX or their precursors remains circumstantial.

Platypus Bay, Queensland, regularly produces ciguateric fish including the piscivorous Spanish mackerel (*Scomberomorus commersoni*) and barracuda (*Sphyraena jello*) (Lewis & Endean, 1983,1984). To reduce the adverse impacts of ciguatera, a ban has been imposed on capture of these species in Platypus Bay. Another common fish, *Pomadasys maculatus* (blotched javelin fish; Fig. 1), can also be toxic in this area (Lewis et al.,1988) and may be a link in the transfer of CTXs to Spanish mackerel and barracuda (Lewis & Sellin,1992). *P. maculatus* are often more toxic than Spanish mackerel and toxic individuals of both species are contaminated with CTX-1, -2 and -3 at similar relative levels (Lewis & Sellin,1992). In this paper we report that *P. maculatus* probably accumulates CTX through feeding on invertebrates (especially the shrimps) living in the macroscopic algae (*Cladophora* sp.) that harbours *G. toxicus* in Platypus Bay.

METHODS

POMADASYS MACULATUS IN PLATYPUS BAY

P. maculatus were captured in Platypus Bay (24° 58'S, 153° 10'E) by line or trawl net in ~15 m of water (Holmes et al., this memoir). Feeding preferences were determined from visual assessment of the stomach and intestinal content of *P. maculatus* collected intermittently over a year (n = 40). Small benthic fish that could be potential prey of *P. maculatus* could not be confirmed by scuba diver observations in Platypus Bay. The

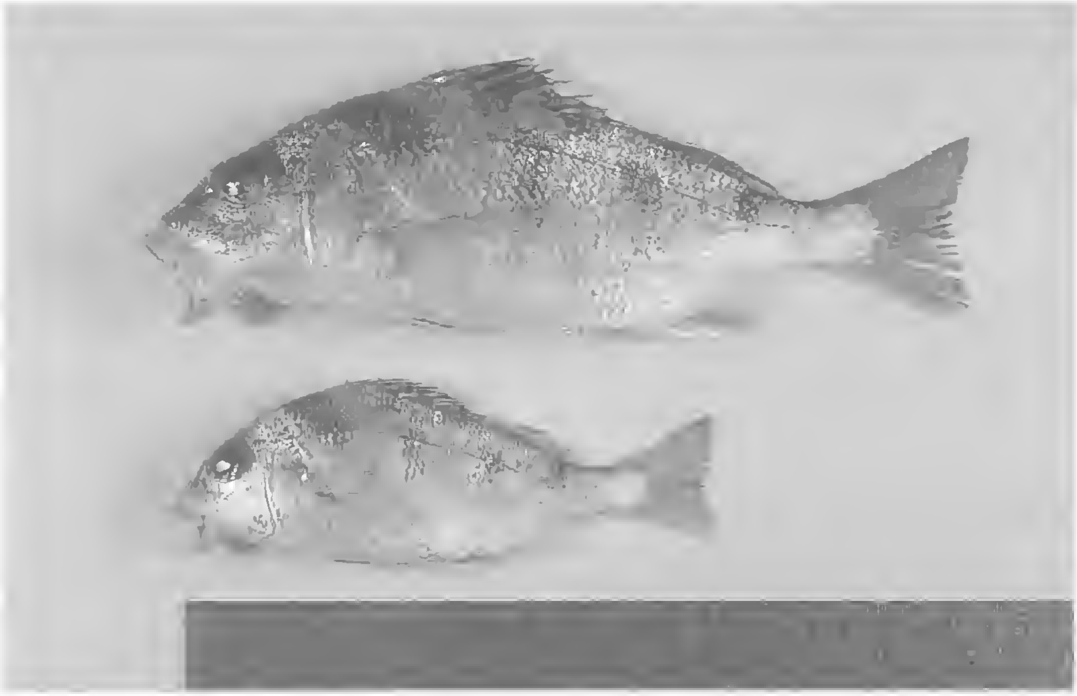

FIG.1. Examples of *Pomadasys maculatus* captured from Platypus Bay. Note the downward deflection of the mouth that indicates this species is specialised for bottom foraging.

relative intestine length and pH (after dilution with water) of pooled stomach and intestinal contents (n=5) were measured to assess the digestive strategy.

INVERTEBRATES FROM PLATYPUS BAY

Invertebrates (alpheid shrimps, crabs, nematodes, polychaetes, gastropods) living in association with the green macroalga, *Cladophora* sp., carpeting the sandy substrate of the study site were collected by small dredge, beam trawl or diver. From 8–10 May 1991 a small dredge was used to collect several species of benthic 'worms', in addition to shrimp and crab samples. The diver-collected (9 May 1988) *Cladophora* (9.2kg) was processed exclusively for the small gastropods present. A beam trawl was used to obtain (22 October 1991 and 20 March 1992) two further *Cladophora* samples of 191 and 154kg from which additional shrimps and crabs were collected and extracted for toxins. The visible invertebrates in each of the above samples were sorted by hand.

ASSESSMENT OF TOXIN LEVELS IN INVERTEBRATES IN PLATYPUS BAY

Invertebrates were extracted for ciguatoxin-like toxins with acetone and the acetone-soluble material partitioned as previously described (Lewis et al.,1992). The ether-soluble and selected butanol-soluble fractions (up to 30mg) were dried, suspended in Tween 60/saline and assayed in 20±2g mice (Quackenbush strain, either sex). Signs in mice (n=2) following intraperitoneal (i.p.) injection of these fractions were used to characterise the toxicity of each fraction (Holmes et al.,1991; Lewis et al.,1991).

RESULTS

Toxicity was detected in the ether-soluble fraction of shrimps but not in the ether-soluble material of other invertebrates (Table 1). Mouse bioassay signs induced by the ether-soluble toxin in the shrimps included severe diarrhoea and laboured respiration, signs consistent with an injection of a sub-lethal dose of ciguatoxin-like toxins. Two additional samples of shrimps (178 and 163g) and crabs (78 and 11g), collected by beam trawl, had levels of gambiertoxins below the limit of detection of the mouse bioassay. The butanol-soluble material from the gastropods as well as from the shrimps and crabs from these latter two collections were also assayed by mouse

TABLE 1. Yield (mg) and toxicity (MU) of ether extracts of invertebrates collected by dredge from Platypus Bay, Queensland.

Invertebrate	Wet Weight (g)	Average Size g (range)	Ether extract mg (MU)[a]
Shrimps (mostly *Alpheus* sp.)	42.5	0.15 (0.02-1.2)	30(0.5)
Crabs (mostly *Thalamita* sp.)	21.9	0.2 (0.04-1.7)	36 (0)
Small nematodes	38.1	0.05	94 (0)
Large tube-dwelling polychaetes	7.9	0.2	27 (0)
Gastropods	-	~0.1	(0)

[a]Ciguatoxin-like activity quantified in mouse units (MU). One MU = 1 LD_{50} dose for a 20 g mouse.

bioassay. Toxins resembling the maitotoxins were detected in the butanol fraction but these were not characterised further.

P. maculatus in Platypus Bay which ranged from 30-300g (fork length 13.5-24.0cm) has a thin walled stomach (pH=7.0) and a relatively short intestine (10-21cm) (pH=6.3). The downward pointing mouth (Fig.1) indicates that this species is a specialised benthic forager. Identifiable stomach contents of *P. maculatus* comprised mostly shrimps and crabs with occasionally some *Cladophora* and small unidentified fish.

DISCUSSION

P. maculatus has a near neutral pH digestive system that would be unlikely to provide the conditions for acid- catalysed spiroisomerisation of CTX-2 (or the putative 52-epi CTX-1 named CTX-4) to CTX-2 and CTX-1, respectively. Thus the CTX-1 and CTX-3 detected in *P. maculatus* flesh (Lewis & Sellin,1992) may arise as an artefact that results from the purification of CTX-2 and -4 on silicic acid supports eluted with acid solvents such as chloroform.

Of the invertebrates inhabiting *Cladophora* beds in Platypus Bay, only shrimps contained detectable levels ciguatoxin-like toxins (Table 1). The toxin levels in shrimps declined to levels below those detectable by mouse bioassay for two subsequent collections. Shrimps (and perhaps crabs) may be a vector in the transfer of gambiertoxins to carnivorous fish. Another possibility is that *P. maculatus* accumulates gambiertoxins from the *G. toxicus* that is ingested along with the small amounts of *Cladophora* ingested incidentally with the invertebrates. This possibility is considered remote since the prominent herbivore in the area (*Siganus spinus*, a species of similar size to *P. maculatus*) consumes almost entirely *Cladophora* and is seldom toxic (unpubl. data). Another possibility is that *P. maculatus* feeds on the dead remains of ciguateric fish.

For shrimps to accumulate gambiertoxins they must be capable of ingesting *G. toxicus*. This appears likely, since brine shrimp have been shown to feed on *G. toxicus* in the laboratory (Kelly et al.,1992). The detection of ciguatoxin-like toxins in shrimps and maitotoxin-like toxins in shrimps and crabs from Platypus Bay indicates that both groups of invertebrates consume *G. toxicus*. Analysis of intestinal content of shrimps (n = 2) revealed a range of detritus similar to or larger than *G. toxicus*, but no *G. toxicus*. This analysis was conducted on shrimps collected at a time when no detectable gambiertoxin could be extracted from these shrimps.

G. toxicus cells have been shown to be toxic to brine shrimps (Kelly et al.,1992). Toxie effects of *G. toxicus* on shrimps may facilitate the selective feeding of fish on shrimps containing the highest toxin levels. Such selective feeding provides a mechanism for funnelling *G. toxicus* toxins, especially gambiertoxins, to *P. maculatus* (Fig.2). It remains to be established if shrimps are capable of biotransforming the gambiertoxins to ciguatoxins or if this capacity is exclusive to fish. The piscivorous fish likely to prey on *P. maculatus* include *Scomberomorus commersoni*, *Sphyraena jello* and *Seriola lalandi* (yellow-tail kingfish).

Environmental and/or genetic factors leading to a proliferation of gambiertoxins and consequent outbreaks of ciguatera remain to be

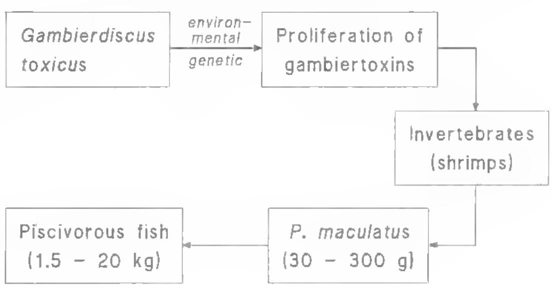

FIG.2. A model for the food chain transfer of ciguatoxins and/or gambiertoxins to *P. maculatus* and piscivorous fish in Platypus Bay. This study has implicated shrimps as a key vector. The size of the fish (kg) involved in this transfer are indicated.

elucidated. Given that *P. maculatus* is at times highly toxic (Lewis & Sellin,1992) and within a year can be non-toxic, it is likely that the gambiertoxins enter the food chain as intense pulses that perhaps last for only several weeks. At these times the shrimps would presumably be highly toxic. Depuration and/or detoxification may account for the apparent rapid loss of gambiertoxins and ciguatoxins from shrimps, crabs and *P. maculatus*.

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